JOURNAL OF ENVIRONMENTAL SCIENCES 155 (2025) 329–342

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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Research Article

Heavy metals are liver fibrosis risk factors in people without traditional liver disease etiologies

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ARTICLE INFO

Article history:
Received 6 February 2024
Revised 20 August 2024
Accepted 21 August 2024
Available online 30 August 2024

Keywords:
Metabolic dysfunction-associated steatotic liver disease (MASLD)
Fibrosis
Transient elastography
Racial disparities
Heavy metal
Cardiometabolic risk factors (CMRFs)

ABSTRACT

Liver fibrosis is an important predictor of mortality. Liver disease case definitions changed in 2023. These definitions include an easily over-looked group with no traditional etiology (NTE) of liver disease and no steatosis. We analyzed heavy metals and cardiometabolic risk factors (CMRFs) as fibrosis risk factors in the NTE group and in people with another newly-defined condition, metabolic dysfunction-associated steatotic liver disease (MASLD). Two National Health and Nutrition Examination Survey (NHANES) datasets were analyzed. In NHANES III (1988–1994), fibrosis and steatosis were defined by Fibrosis-4 scores and ultrasound, respectively, in 12,208 adults. In NHANES 2017–2020, fibrosis and steatosis were defined by transient elastography and the controlled attenuation parameter (CAP) in 5525 adults. Fibrosis risk factors varied over time and by race/ethnicity. In the earlier dataset, NTE-fibrosis had a positive, non-significant, association with high blood levels of lead (Pb). MASLD-fibrosis was associated with Pb (OR = 2.5, 95 % CI, 1.4–4.4) and not with CMRFs in non-Hispanic Blacks but was associated with CMRFs in non-Hispanic Whites. Heavy metal exposures fell between the two time periods. In the later dataset, NTE-fibrosis was associated with Pb (OR = 4.2, 95 % CI, 2.6–6.8) and cadmium (OR = 1.8, 95 % CI, 1.1–3.0) in the total population, but not with most CMRFs. MASLD-fibrosis was strongly-significantly associated with CMRFs in every racial/ethnic group except non-Hispanic Blacks in whom CMRFs were only weakly associated with MASLD-fibrosis. Heavy metal pollution, which disproportionately impacts minoritized populations, decreased over time, but remained strongly associated with liver fibrosis in people lacking traditional etiological factors for liver disease.

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https://doi.org/10.1016/j.jes.2024.08.027

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Introduction

Most liver disease is caused by some type of exogenous toxic exposure, either exposure to a hepatitis virus, excess alcohol, diet that results in obesity and elevated cardiometabolic risk, and/or environmental toxins, including heavy metals (Barouki et al., 2023; Jan et al., 2015; Singh et al., 2024). Over time, the nature of toxic exposures and their relative intensities have changed. Recently, the global increase in obesity and other cardiometabolic risk factors (CMRFs) has caused a steep rise in steatotic liver disease that is part of a growing public health crisis. In 2023, members of multinational liver societies and patient advocacy groups defined a new condition, metabolic dysfunction-associated steatotic liver disease (MASLD) (Rinella et al., 2023a). The MASLD case definition requires at least one of five clearly-specified CMRFs plus steatosis (excess accumulation of intrahepatic lipid) (Rinella et al., 2023a). In this study, we focus on people with MASLD and people with no traditional etiology (NTE) of liver disease. The NTE group includes people without viral hepatitis (VH), alcohol-associated liver disease (ALD), or MASLD. We reasoned that the absence of other causes of liver injury in the NTE group will enable the hepatotoxic effects of environmental pollutants to be more easily observed.

Fibrosis is the most important predictor of liver-related morbidity and mortality (Dulai et al., 2017; Lim and Kim, 2008). Lead (Pb) and cadmium (Cd) exposures are associated with liver fibrosis (Ma et al., 2017; Reja et al., 2020; Xu et al., 2021; Zhang et al., 2022). Among people with non-alcoholic fatty liver disease (NAFLD) defined by the fatty liver index, those with elevated blood levels of Pb had a six-fold increase in severe liver fibrosis, as indicated by a NAFLD Fibrosis Score $>$ 0.676 (Reja et al., 2020). In isolated rat liver mitochondria, Pb reduced aerobic respiration and increased oxidative stress (Ma et al., 2017). Chronic dietary Cd exposure upregulated expression of TGF-β1, collagen-1, and tissue inhibitor of metalloproteinase-1 and accelerated liver fibrosis in a murine model (Xu et al., 2021). In vitro, Cd exposure increased steatosis and fibrosis of a human bipotent progenitor cell line HepaRG and hepatocellular carcinoma cell lines (HepG2 and SK-Hep1) by activating Notch and AKT/mTOR signaling pathways (Niture et al., 2023).

Cd and Pb have become significant environmental pollutants as a result of their use in a wide variety of commercial products including paints, pipes, and batteries. Children are particularly vulnerable to poisoning from environmental Pb (Mitra et al., 2022). Toxic workplace and environmental exposures are unevenly distributed across the multiracial population of the United States and are often higher among people of color (Adams et al., 2023; Jones et al., 2022; Juon et al., 2021). Additionally, it is likely that there is individual variation in susceptibility to toxic injury. In our previous study (Ma et al., 2023a), high blood levels of Pb were associated with advanced liver fibrosis exclusively in non-Hispanic Blacks (NHB). This increased susceptibility to Pb-mediated liver injury may have a parallel in susceptibility to smoking-related lung cancer. African Americans develop lung cancer at younger ages and with fewer smoking pack-years than to others (Prosper et al., 2020). African Americans' vulnerability to Pb exposure may be due to higher levels of Pb exposure during a critical developmental period in combination with genetic, medical, and sociodemographic factors.

The primary aim of this study was to investigate heavy metals (Pb and Cd) and CMRFs as fibrosis risk factors in people in the NTE and MASLD categories using two consecutive National Health and Nutrition Examination Survey (NHANES) datasets. Our study examines changes over time and contrasts with previous studies using these data sources. Previous studies of metal toxicity did not use imaging data to define steatosis (Li et al., 2023; Reja et al., 2020), did not classify liver disease according to the current nomenclature (Reja et al., 2020; Zhang et al., 2022), did not stratify fibrosis risk factors by race/ethnicity, did not investigate the NTE group, and did not determine how the criteria used to define steatosis impact the size and composition of the MASLD and NTE groups. Our most important findings are: 1) that associations between exposures and fibrosis vary by race/ethnicity and 2) that Pb and Cd are associated with fibrosis in the NTE group in the more recent dataset (2017–2020), although blood levels of both heavy metals have decreased over time.

1. Materials and methods

1.1. *Study population and data sources*

Data collection in NHANES follows standardized procedures approved by the National Center for Health Statistics Research Ethnic Review Board. IRB review was waived for analysis of de-identified NHANES data (Johnson et al., 2013). The NHANES III (1988–1994) dataset with abdominal ultrasound data and NHANES 2017-March 2020 datasets with vibration controlled transient elastography (VCTE) data were used. The public-use linked mortality file was obtained through 2019 (NCHS, 2019).

1.2. *Indicators of steatotic liver disease (SLD) and significant fibrosis*

In NHANES III, SLD was defined by ultrasound and classified as mild, moderate, or severe and fibrosis ($\geq$ F1) was estimated using the Fibrosis-4 (FIB-4) score, with the following age-specific thresholds: $\geq$ 1.05 (Ishiba et al., 2018) for adults 20 $\leq$ age $\leq$ 35 yr, FIB-4 $\geq$ 1.3 for 35 $<$ age $<$ 65 yr, and FIB-4 $\geq$ 2.0 for age $\geq$ 65 yr (McPherson et al., 2017). Age-specific cut points have 90 % (Ishiba et al., 2018) sensitivity for individuals below 30 years of age and 70 % (McPherson et al., 2017) specificity for individuals over 65 years of age, which is more accurate than FIB-4 $\geq$ 1.3, the cut point used to define this group in American Gastroenterology Association (AGA) guidelines (Kanwal et al., 2021). In sensitivity analyses FIB-4 $\geq$ 2.67 was used to define clinically-significant fibrosis ($\geq$ F2). In NHANES (2017 – March 2020), SLD was defined by VCTE and the controlled attenuation parameter (CAP) scores ranging from $\geq$ 240 dB/m to $\geq$ 300 dB/m. Clinically-significant ($\geq$ F2) fibrosis was defined by liver stiffness $\geq$ 8.6 kPa (Siddiqui et al., 2019).

1.3. *MASLD-defining CMRFs*

The following CMRFs were defined by the nomenclature panel (Rinella et al., 2023a): 1) Elevated adiposity, body mass

index (BMI) ≥ 25 kg/m^2 or waist circumference (WC) > 80 cm (women) and > 94 cm (men); 2) Prediabetes/diabetes, fasting plasma glucose (FPG) ≥ 100 mg/dL and/or hemoglobin A1c (HbA1c) ≥ 5.7 % and/or self-reported diagnosis of diabetes and treatment; 3) Elevated blood pressure, systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg and/or use of anti-hypertensive medication; 4) Elevated triglycerides, plasma triglycerides ≥ 150 mg/dL and/or self-reported lipid lowering treatment; 5) Reduced plasma high-density lipoprotein cholesterol (HDL), plasma HDL ≤ 50 mg/dL (women) and ≤ 40 mg/dL (men) and/or self-reported lipid lowering treatment.

1.4. *Liver disease categories*

As per the new diagnostic algorithm, MASLD was defined as SLD with at least one CMRF and consumption of < 140 g/wk of alcohol for women and < 210 g/wk for men and no other discernible cause of liver disease. MetALD was defined as MASLD with increased alcohol intake, 140–350 g/wk of alcohol for women and 210–420 g/wk of alcohol for men over the past 12 months. ALD was > 350 g/wk of alcohol for women or > 420 g/wk for men over the past 12 month. VH was past/current infection with hepatitis B virus (HBV), positive core antibody or surface antigen; or hepatitis C virus (HCV), RNA or antibody. Cryptogenic SLD was SLD without a CMRF or any other identifiable cause. NTE was no VH, ALD, or MASLD, and no SLD.

1.5. *Demographic variables*

Self-reported sex (male/female) and race/ethincity (non-Hispanic White: NHW; non-Hispanic Black: NHB; Mexican American: MA; other race: O (including non-MA Hispanics and others)) were included. In NHANES 2017-March 2020 with VCTE data, the age range was 20 to over 80 yr (people older than 80 were coded as 80 yr according to NHANES). In NHANES III, participants with ultrasound data ranged 20–74 yr.

1.6. *Risk factors ≥ F1 fibrosis*

Variables considered for analysis were the five MASLD-defining CMRFs and additional factors defined as in our previous study (Ma et al., 2023a). Briefly, diabetes was HbA1c ≥ 6.5 %, and/or FPG ≥ 126 mg/dL and/or self-reported. Past/current smokers answered “Yes” to the question “Have you smoked at least 100 cigarettes in your lifetime?”; never-smokers answered “No”. The responses to questions about alcohol consumption were used to create three mutually exclusive groups, lifetime abstainers (answered “No” for the question of “Ever had a drink of any kind of alcohol”), former drinkers (had drinks in their lifetime but none in the past year), current drinkers (had drinks in the past year). Blood levels of Pb and Cd were measured in NHANES 2017–2020 cycle; Blood levels of Pb and urine levels of Cd were measured in NHANES III. Blood levels of Pb and blood/urine levels of Cd were analyzed as continuous and binary variables (quartiles (Q)1–3 versus Q4). Poverty was defined as a family poverty-income-ratio below 1.0 (Ma et al., 2023a).

1.7. *Statistical analysis*

Analyses followed NHANES guidelines (Johnson et al., 2013). Data analysis accounted for the complex NHANES design by using survey commands in SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Age standardized estimates were calculated using the direct method and standardized to the 2000 US census population. Differences between groups were tested by t statistics or Mann-Whitney U test (Hales et al., 2020). To determine the population counts in each etiology group, the weighted prevalence was calculated and subsequently multiplied by the estimated population in the United States obtained from the American Community Survey (Johnson et al., 2013). Univariable and multivariable survey logistic (MVL) regression with appropriate sample weights were used to examine fibrosis risk factors. Survey-weighted adjusted univariable and multivariable Cox proportional hazards models were used to investigate the association between liver disease categories and all-cause mortality. Missing values of covariables <10 % were estimated using multivariable imputation by chained equations (Yuan, 2010). Combined estimates using ten imputed datasets were calculated. Statistical significance was taken as a two-sided P value <0.05.

2. Results

2.1. *Projecting the liver disease diagnostic algorithm onto two consecutive NHANES datasets*

The new algorithm for liver disease diagnosis and classification was projected onto nationally representative datasets from two time periods (Fig. 1a and b). Steatosis was determined by ultrasound in the NHANES III dataset (1988–1994) and by CAP in the NHANES (2017–2020) dataset. In the earlier time-period, the age-standardized weighted prevalence of SLD was 34.0 % (95 % CI, 31.5 %–36.6 %) (Fig. 2a). In the later period, the prevalence was 62.1 % (95 % CI, 59.8 %–64.3 %) when 240 dB/m, the supplier (EchoSens)-recommended cut point for detecting steatosis grade 1 or higher (De Lédinghen et al., 2017), was used. At a cut point of 285 dB/m, which optimizes sensitivity and specificity for differentiating steatosis grade 0 from 1 to 3 based on the Youden’s index (Siddiqui et al., 2019), the prevalence was 36.3 % (95 % CI, 34.1 %–38.5 %) (Fig. 2b). As the CAP threshold increased from ≥ 240 dB/m to ≥ 300 dB/m, the percentage of the population in the NTE category increased from a weighted prevalence of 32.1 % (95 % CI 30.1 %- 34.2 %) to 61.8 % (95 % CI 59.4 %–64.3 %). Increased adiposity (obesity) was the most prevalent CMRF during both time periods (Appendix A Table S1). It increased about 15 % between them (Appendix A Table S2).

2.2. *Assessing risk factors for liver fibrosis*

2.2.1. *Analysis of the NHANES III (1988–1994) dataset*

2.2.1.1. *Risk factors for NTE-associated fibrosis (NTE-fibrosis)*

Fibrosis risk factors were examined in the 6595 participants in the NTE group (Fig. 1a). Age-specific FIB-4 cut points were

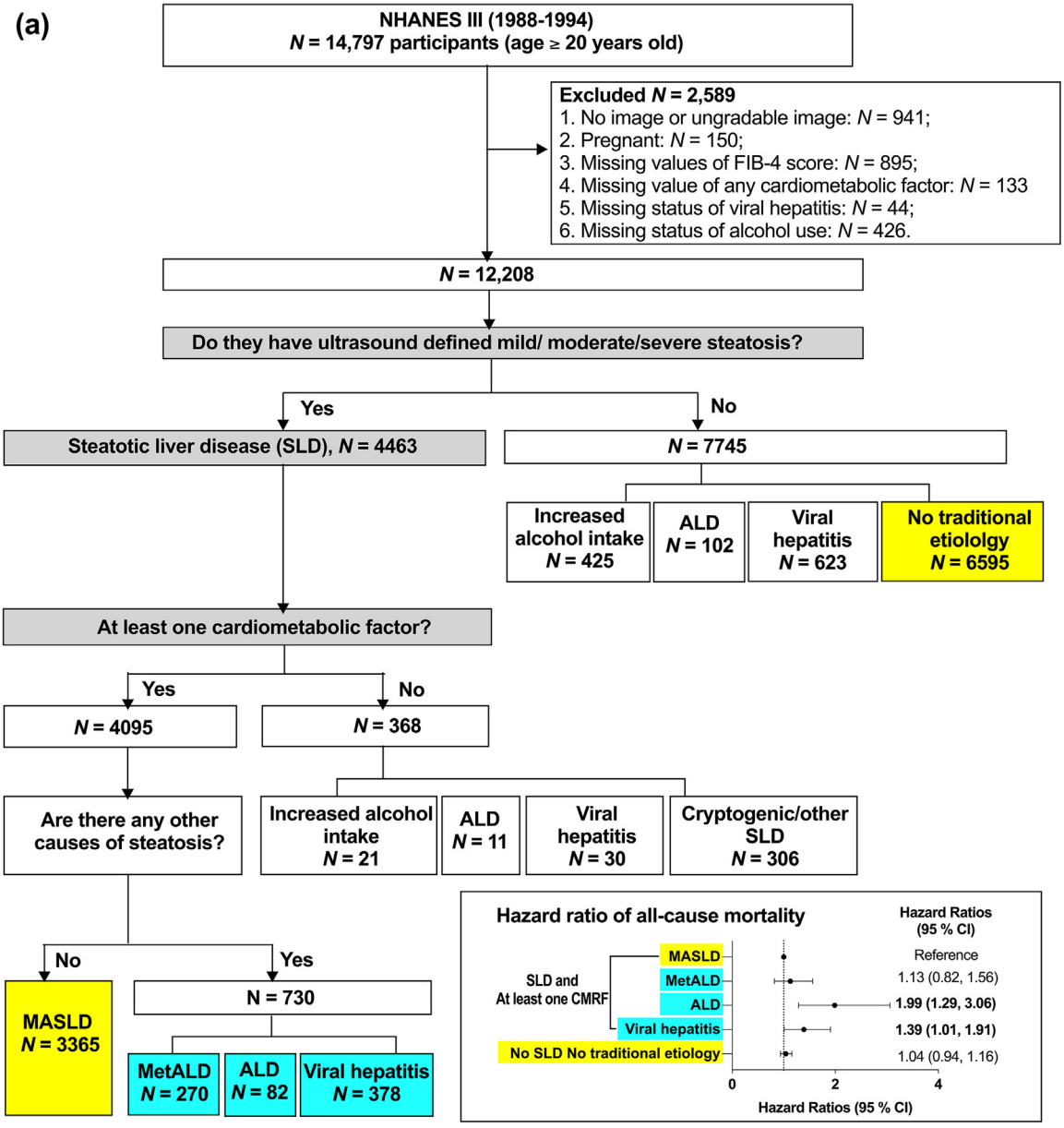
(a)
NHANES III (1988-1994)
N = 14,797 participants (age ≥ 20 years old)
Excluded N = 2,589
1. No image or ungradable image: N = 941;
2. Pregnant: N = 150;
3. Missing values of FIB-4 score: N = 895;
4. Missing value of any cardiometabolic factor: N = 133
5. Missing status of viral hepatitis: N = 44;
6. Missing status of alcohol use: N = 426.
N = 12,208
Do they have ultrasound defined mild/ moderate/severe steatosis?
Yes
No
Steatotic liver disease (SLD), N = 4463
N = 7745
Increased alcohol intake N = 425
ALD N = 102
Viral hepatitis N = 623
No traditional etiololgy N = 6595
At least one cardiometabolic factor?
Yes
No
N = 4095
N = 368
Are there any other causes of steatosis?
Increased alcohol intake N = 21
ALD N = 11
Viral hepatitis N = 30
Cryptogenic/other SLD N = 306
No
Yes
MASLD N = 3365
N = 730
MetALD N = 270
ALD N = 82
Viral hepatitis N = 378
Hazard ratio of all-cause mortality
Hazard Ratios (95 % CI)
SLD and At least one CMRF
MASLD
MetALD
ALD
Viral hepatitis
No SLD No traditional etiology
Reference
1.13 (0.82, 1.56)
1.99 (1.29, 3.06)
1.39 (1.01, 1.91)
1.04 (0.94, 1.16)
0
2
4
Hazard Ratios (95 % CI)

Fig. 1 – The new nomenclature's diagnostic algorithm applied to adults in the United States with steatosis defined by abdominal ultrasonography in NHANES III and by VCTE-CAP in NHANES 2017–2020. (a) Flowcharts of 14,797 adults (20–74 years old) in NHANES III (1988–1994) and a forest plot (insert) of hazard ratios of all-cause mortality compared between people with MASLD and people with other diagnoses: MetALD/SLD/CMRF, ALD/SLD/CMRF, viral hepatitis/SLD/CMRF, and No-SLD/No Traditional Etiology; (b) Flowcharts of 14,300 adults (age ≥ 20 years old) in NHANES 2017-March 2020 categorized according to the new nomenclature. SLD in NHANES III was defined by ultrasound measurements of mild/moderate/severe steatosis, while in NHANES 2017-March 2020, and it was defined by VCTE-CAP ≥ 240 dB/m. ALD: alcohol associated liver disease; CAP: controlled attenuation parameter; CMRF: cardiometabolic risk factor; NHANES: National Health and Nutrition Examination Survey; MetALD: metabolic dysfunction-associated alcohol-associated liver disease; MASLD: metabolic dysfunction-associated liver disease; SLD: steatotic liver disease; VCTE: vibration controlled transient elastography.

used to define intermediate-to-high fibrosis risk (≥ F1). In multivariable logistic regression (MVL) models that adjusted for age, sex, smoking status, alcohol use, and poverty, no CMRF had a positive and statistically significant association with fibrosis risk in any racial/ethnic group. A high (4th quartile) blood level of Pb was positively associated with fibrosis among NHB persons but the relationship did not meet standard criteria of statistical significance (odds ratio (OR) = 1.21, 95 % CI: 0.71–2.04) (Appendix A Fig. S1). In a sensitivity analysis with the outcome of clinically significant fibrosis (≥ F2), defined as FIB-4 ≥ 2.67, a high (4th quartile) blood level of Pb had a higher OR (1.56, 95 % CI: 0.72–3.36) than any CMRF (Appendix A Fig. S2). Sample size limitations precluded stratified by race/ethnicity.

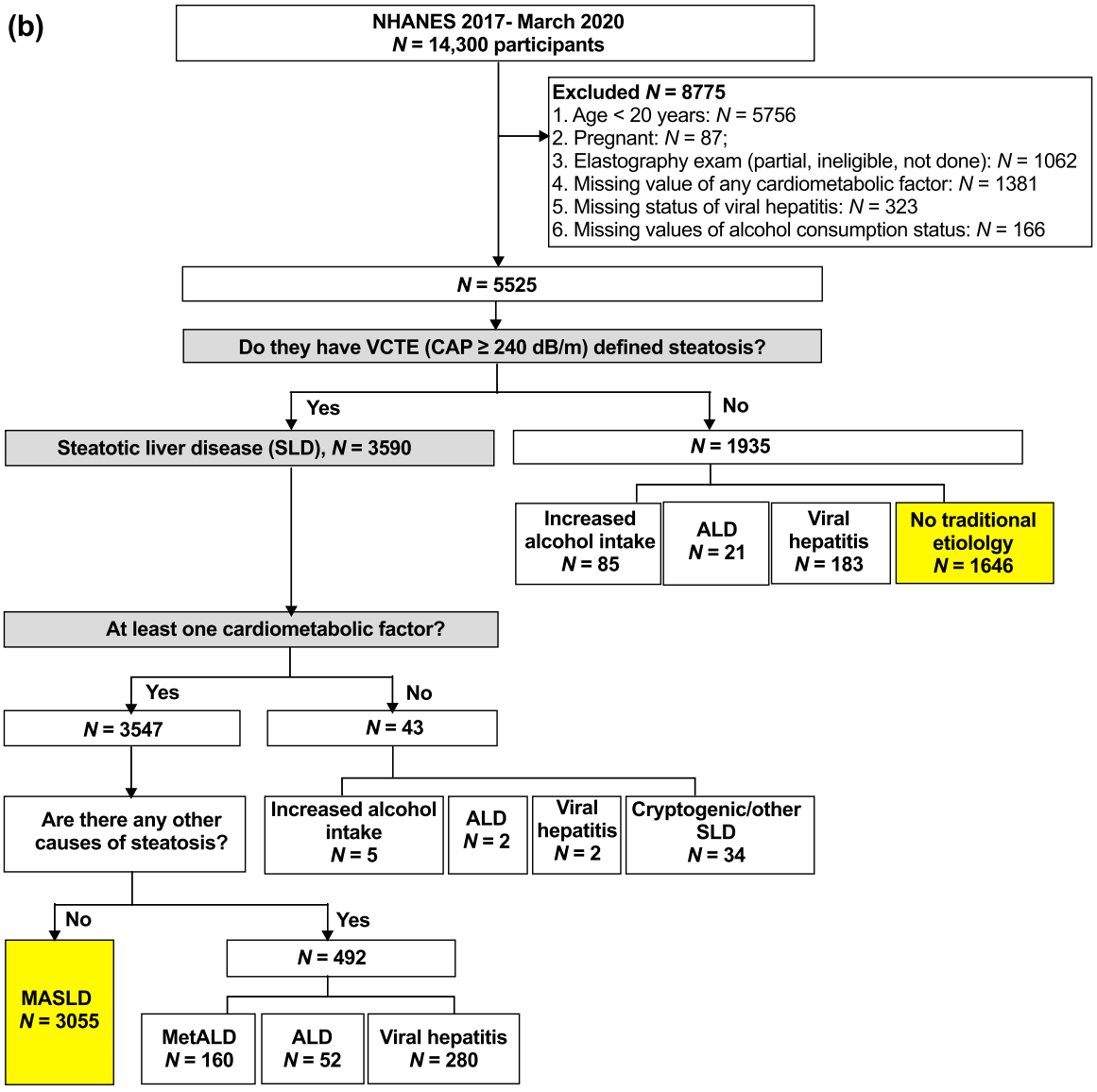
(b)
NHANES 2017- March 2020
N = 14,300 participants
Excluded N = 8775
1. Age < 20 years: N = 5756
2. Pregnant: N = 87;
3. Elastography exam (partial, ineligible, not done): N = 1062
4. Missing value of any cardiometabolic factor: N = 1381
5. Missing status of viral hepatitis: N = 323
6. Missing values of alcohol consumption status: N = 166
N = 5525
Do they have VCTE (CAP ≥ 240 dB/m) defined steatosis?
Yes
No
Steatotic liver disease (SLD), N = 3590
N = 1935
Increased alcohol intake N = 85
ALD N = 21
Viral hepatitis N = 183
No traditional etiololgy N = 1646
At least one cardiometabolic factor?
Yes
No
N = 3547
N = 43
Are there any other causes of steatosis?
Increased alcohol intake N = 5
ALD N = 2
Viral hepatitis N = 2
Cryptogenic/other SLD N = 34
No
Yes
MASLD N = 3055
N = 492
MetALD N = 160
ALD N = 52
Viral hepatitis N = 280

Fig. 1 – Continued

2.2.1.2. Risk factors for MASLD-fibrosis As in the analysis of the NTE group, age-specific FIB-4 cut points were used to define intermediate-to-high fibrosis risk (≥ F1). Reduced HDL was the only CMRF associated with MASLD-fibrosis in the total population in MVL models that adjusted for age, sex, smoking status, alcohol use, and poverty. Reduced HDL was also a MASLD-fibrosis risk factor among NHW persons (OR = 1.67, (95 % CI, 1.27–2.21)) (Fig. 3). Compared to other racial/ethnic groups, NHB persons had a distinctive set of MASLD-fibrosis risk factors. In MVL models, no CMRF was significantly associated with MASLD-fibrosis in NHB; the OR for reduced HDL was 0.88 (95 % CI, 0.51–1.51). In contrast, high (4th quartile) blood levels of Pb had an OR of 2.53 (95 % CI, 1.44–4.45) (Fig. 3, Appendix A Table S3). Pb was not a MASLD-fibrosis risk factor in the total population (OR = 1.04, (95 % CI, 0.66–1.65)) or in any racial/ethnic group other than NHB. Sensitivity analysis showed that elevated blood pressure was the only CMRF associated with ≥ F2 fibrosis in the total population (Fig. 3, Appendix A Table S4). Sample size limitations precluded an analysis of risk factors for ≥ F2 fibrosis stratified by race/ethnicity.

2.2.2. Analysis of the later NHANES (2017-March 2020) dataset

2.2.2.1. Risk factors for NTE-fibrosis Risk factors for ≥ F2 in the NTE category were analyzed using a liver stiffness measurement of ≥ 8.6 kPa. Because the size and composition of the NTE group depends on the CAP threshold used to define steatosis, a series of analyses were performed using different CAP thresholds. When the NTE group was defined as CAP < 240 dB/m, high blood levels of Pb and Cd were associated with ≥ F2 fibrosis in MVL models that adjusted for age and sex. Of the five CMRFs in the MASLD case definition, only elevated triglycerides was a fibrosis risk factor (Fig. 4a-f). The ORs, for high (4th quartile vs 1st-3rd quartiles) blood levels of Pb and Cd were 4.17 (95 % CI, 2.56–6.78) and 1.84

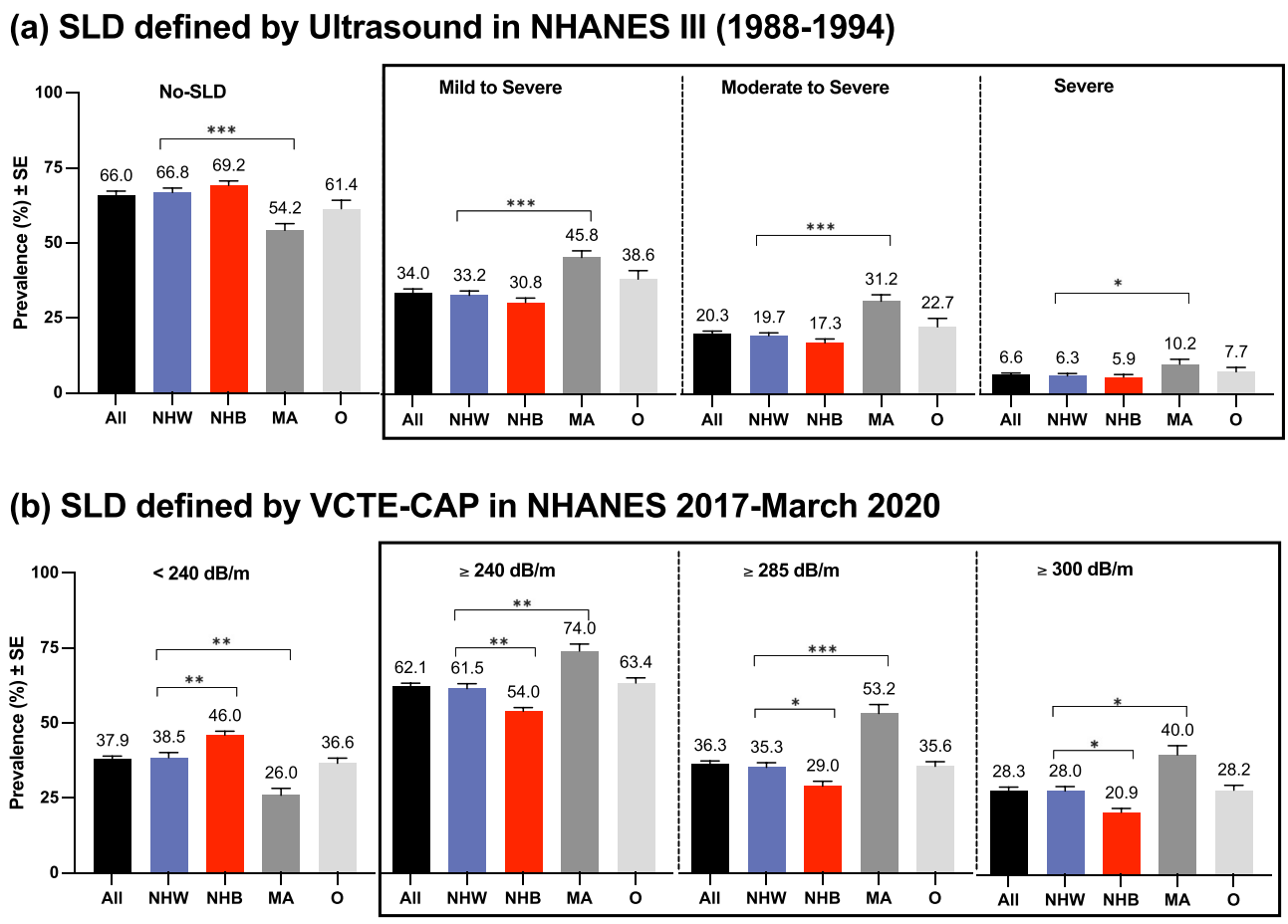

Fig. 2 – The age-standardized weighted percentage of SLD, stratified by race/ethnicity, with SLD defined by ultrasound categories (a) and VCTE-CAP thresholds (b). Ultrasound categories were No-SLD, mild to severe steatosis, moderate to severe steatosis, and severe steatosis in the total cohort (black), non-Hispanic White (NHW, blue), non-Hispanic Black (NHB, red), Mexican American (MA, grey) and other (O, light grey) racial/ethnic groups. VCTE-CAP categories were < 240 dB/m, ≥ 240 dB/m, ≥ 285 dB/m, and ≥ 300 dB/m. Differences between groups were tested by univariate t statistic. Statistical significance was a two-sided P value <0.05. *P < 0.05, **P < 0.001, ***P < 0.0001. SE: standard error.

(95 % CI, 1.14–2.98), respectively (Fig. 4 g and h, Appendix A Table S5). When the NTE group was defined using CAP < 285 dB/m, the NTE group was larger and high blood levels of Pb and Cd were associated with fibrosis in univariable logistic regression models (Appendix A Table S6). In MVLs that adjusted for age and sex, high Cd remained a statistically-significant risk factor, with an OR of 1.86 (95 % CI, 1.11–3.11), while high Pb had an OR of 1.61 (95 % CI, 0.97–2.66, $p = 0.065$) (Fig. 4).

2.2.2.2. *Risk factors for MASLD-fibrosis* MASLD-fibrosis risk factors varied significantly by race in MVL models that adjusted for age, sex, smoking status, alcohol use, and poverty and varied with the CAP threshold. At the supplier (EchoSens)-recommended threshold of ≥ 240 dB/m, neither prediabetes/diabetes nor elevated blood pressure was significantly associated with ≥ F2 fibrosis in NHB, while both were risk factors in all other racial/ethnic groups (Appendix A Fig. S3 and Table S7). The ORs for diabetes ranged from 1.54 (95 % CI, 0.83–2.87) in NHB to 5.34 (95 % CI, 3.54–8.05) in NHW. The lack of a significant association between diabetes and ≥ F2 fibrosis in NHB (Appendix A Fig. S3c) is consistent with findings in the NHANES III dataset in which risk factors for ≥ F1 fibrosis were examined (Fig. 3), highlighting the distinctiveness of fibrosis risk factors in NHB. Generally similar results were obtained using the CAP threshold of ≥ 285 dB/m (Appendix A Fig. S4 and Table S8).

2.3. *Decline in blood Pb levels: NHANES III (1988–1994) to 2017–2020*

Blood levels of Pb were available in the NHANES III and NHANES 2017–2020 datasets. The Mann-Whitney test was used to compare the distribution of blood Pb levels between these two periods. Pb levels were significantly higher in NHANES III than in NHANES 2017–2020 across the total population and within the NTE and MASLD groups (Fig. 5). Pb levels decreased approximate 3-fold over 30 years (Appendix A Table S9).

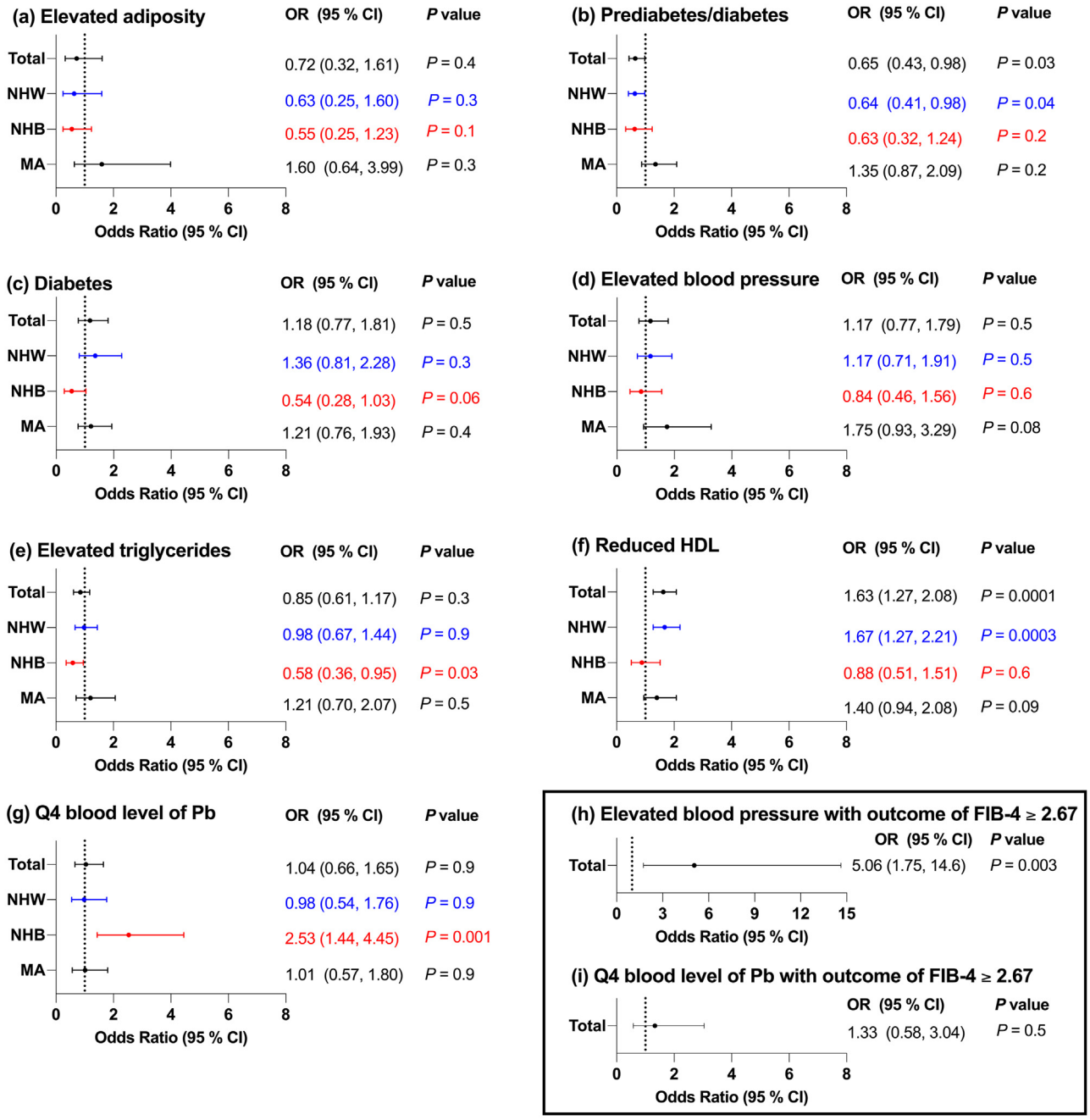

Fig. 3 – Odds ratios from multivariable logistic regression models with the outcome of MASLD-fibrosis stratified by race/ethnicity in the NHANES III dataset with abdominal ultrasound measurements used to identify participants with steatosis. In panels (a) to (g), fibrosis among MASLD cases was defined by age-specific FIB-4 scores (≥ F1), while in panels (h) and (i), fibrosis was defined by FIB-4 ≥ 2.67 (≥ F2). Models adjusted for age, sex, smoking status, alcohol use and poverty. For the outcome of fibrosis ≥ F1, adjusted odds ratios for independent variables were: (a) elevated adiposity, (b) prediabetes/diabetes, (c) diabetes, (d) elevated blood pressure, (e) elevated triglycerides, (f) reduced HDL, and (g) quartile (Q) 4th blood levels of lead (Pb), in the total population or stratified by race/ethnicity. For the outcome of FIB-4 ≥ 2.67 (≥ F2) (boxed), adjusted odds ratio for independent variables were: (h) elevated blood pressure and (i) Q4 blood levels of Pb in the total cohort. CI: confidence interval; FIB-4: fibrosis-4 score; OR: odds ratio; Q: quartile.

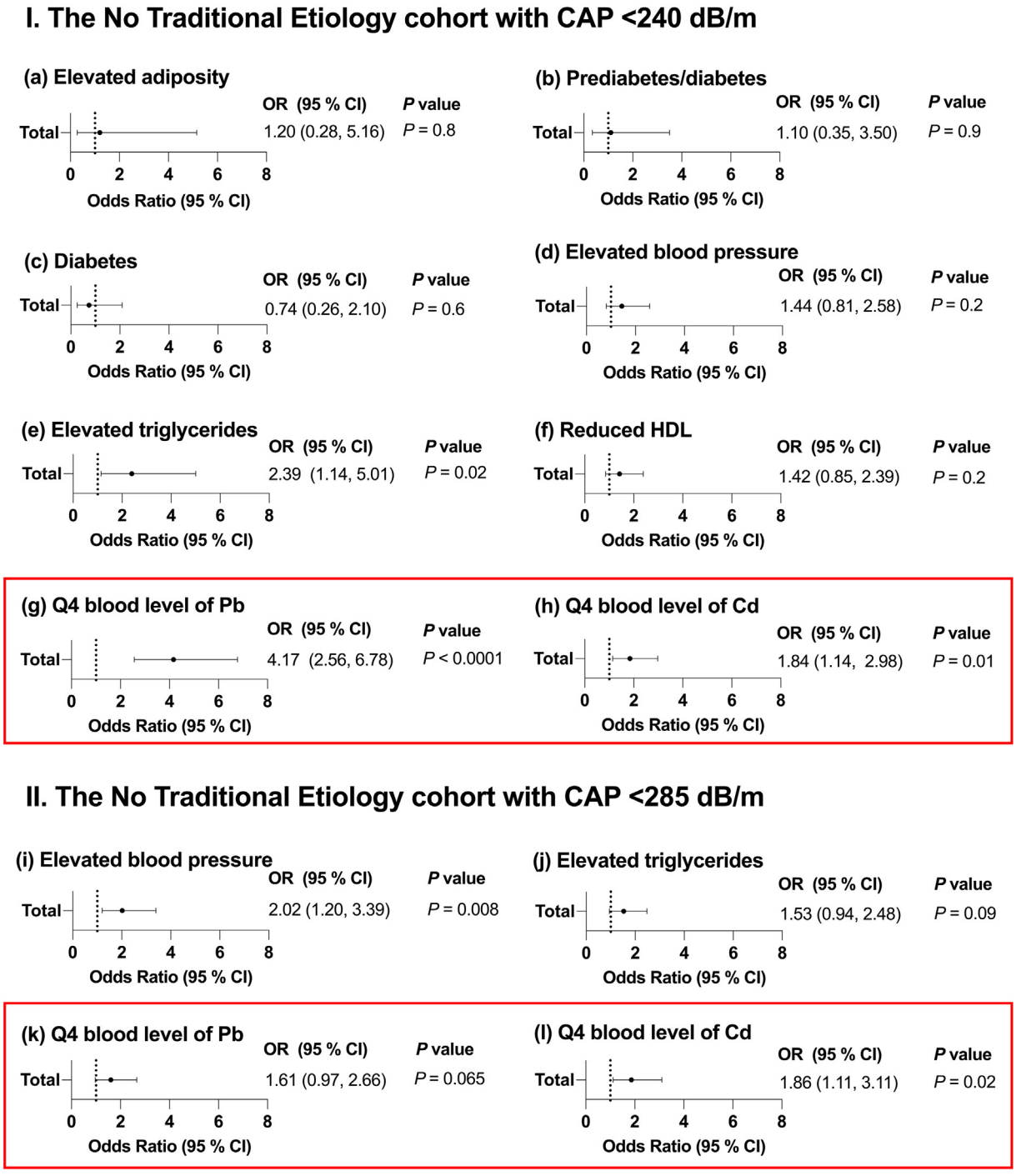

Fig. 4 – Odds ratios from multivariable logistic regression models with the outcome of significant fibrosis in the No Traditional Etiology (NTE) group with the absence of steatosis defined as CAP < 240 dB/m or CAP < 285 dB/m in NHANES 2017-March 2020. Multivariable logistic regression models adjusted for age and sex. In Panel I, the NTE cohort has CAP < 240 dB/m; ORs are: (a) elevated adiposity, (b) prediabetes/diabetes, (c) diabetes, (d) elevated blood pressure, (e) elevated triglycerides, (f) reduced HDL, and Q4 blood levels of (g) Pb and (h) Cd. In Panel II, the NTE cohort has CAP < 285 dB/m; ORs are: (i) elevated blood pressure, (j) elevated triglycerides, and Q4 blood levels of (k) Pb and (l) Cd. Cd: Cadmium.

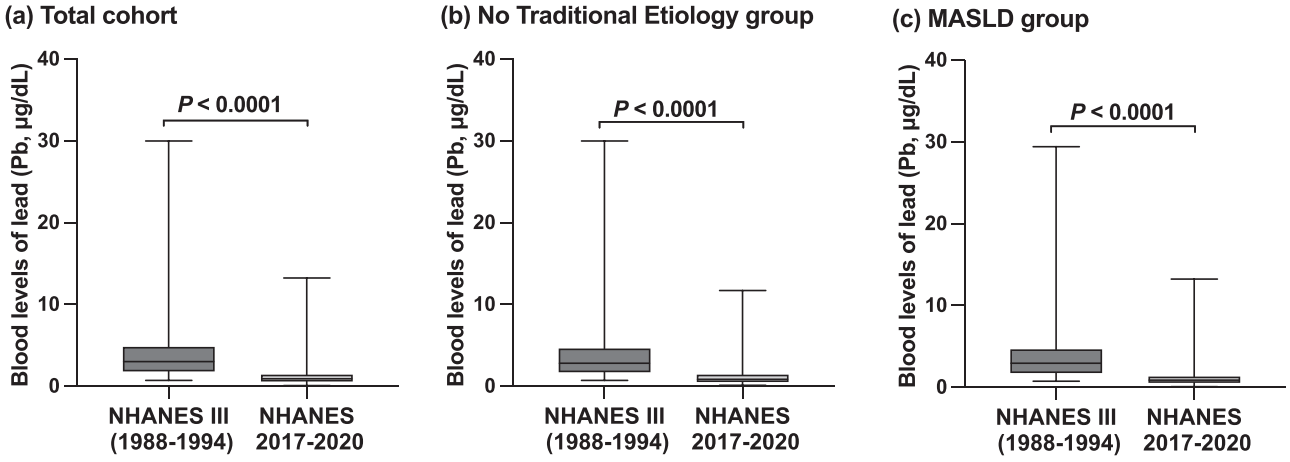

Fig. 5 – Comparison of blood lead (Pb) levels between NHANES III and NHANES 2017–2020 datasets. Blood levels of Pb were compared between NHANES III (1988–1994) and NHANES 2017–2020 using the Mann-Whitney test. Significance was determined at a two-sided $P < 0.05$. To determine the blood levels of Pb in the MASLD and NTE groups, steatosis assessments were conducted as follows: In NHANES III, steatosis was defined through ultrasound measurements indicating mild, moderate, or severe steatosis; in NHANES 2017–2020, steatosis was defined by CAP score ≥ 240 dB/m.

2.4. *Distribution liver diseases underlying liver fibrosis*

2.4.1. *Liver disease etiologies underlying fibrosis in NHANES III: 1988–1994*

The new case-finding algorithm was used to rank liver disease categories based on their population-level contribution to ≥ F2 fibrosis, defined as FIB-4 ≥ 2.67. The NTE group comprised 57.4 % (95 % CI, 55.1 %–59.6 %) of the total population and accounted for the highest percentage of fibrosis cases, 41.8 % (95 % CI, 31.5 %–52.1 %). MASLD accounted for the second highest percentage of fibrosis cases, 22.3 % (95 % CI, 15.9 %–28.7 %) (Fig. 6).

2.4.2. *Liver disease etiologies underlying fibrosis in NHANES: 2017-March 2020*

The proportion of ≥ F2 fibrosis, defined as VCTE liver stiffness ≥ 8.6 kPa (Siddiqui et al., 2019), was analyzed across a range of CAP thresholds. A range was examined because the criteria for diagnosing steatosis are unspecified. At a threshold of 240 dB/m, MASLD accounted for the highest percentage of fibrosis cases, 73.7 % (95 % CI, 67.8 %–79.7 %) (Fig. 7). The NTE group accounted for the second highest percentage, 8.7 % (95 % CI, 5.6 %–11.7 %); viral hepatitis/SLD/CMRF accounted for the third (Fig. 7). At a threshold of 285 dB/m, which was used in a previous study (Vilar-Gomez et al., 2023), MASLD again accounted for the highest percentage of fibrosis cases, 66.4 % (95 % CI, 60.1 %–72.7 %) (Fig. 7) and NTE accounted for the second highest 16.0 % (95 % CI, 12.1 %–19.9 %). As the CAP threshold increased from 240 to 300 dB/m, the number of people with ≥ F2 fibrosis in the MASLD category decreased and the number of people with ≥ F2 fibrosis in the NTE category increased from 1.6 million (95 % CI: 0.8–2.4 million) to 4.6 million (95 % CI: 4.7–6.1 million) (Appendix A Fig. S5). Thus, as the criteria for defining steatosis become more stringent, the number of people in the MASLD group decreases and the fraction of fibrosis attributable to MASLD decreases. Concomitantly, the fraction of fibrosis cases that are in the NTE group increases. It is important to be cognizant of this effect because people in the NTE group may be overlooked by many liver disease screening programs.

AGA guidelines recommend that adults with liver stiffness ≥ 8.0 kPa should be referred to hepatology (Rinella et al., 2023b). The estimated average number of adults in the United States at the time of the NHANES survey in 2017–2020 was 240.4 million (Response Rates and Population Totals, 2022) Over 10 million adults with SLD qualified for hepatology referral and for treatment for steatotic liver disease. In the NTE category, 2.1 to 6.0 million adults qualified for hepatology referral and 1.3 to 3.9 million adults qualified for treatment for liver disease (Appendix A Fig. S5). These large numbers highlight the importance of the NTE category.

2.5. *Mortality in NHANES III: 1988–1994*

The NHANES III (1988–1994) dataset is linked to the National Death Index through December 31, 2019, allowing mortality risk factors to be assessed with about 30 years of follow-up. There was no significant difference in all-cause mortality between the NTE and MASLD groups (Fig. 1a). Among people with SLD and at least one CMRF, the VH and ALD groups had significantly higher all-cause mortality than the MASLD group, with HR = 1.39 (95 % CI, 1.01–1.91) and HR = 1.99 (95 % CI, 1.29–3.06), respectively. MASLD and MetALD groups had similar mortality as each other (Fig. 1a).

3. Discussion

3.1. *Interpretation of the new diagnostic algorithm*

In 2023 a new nomenclature and a new diagnostic algorithm for liver diseases were introduced. These changes were in response to the dramatic increase in the percentage of the population with liver steatosis, which now afflicts over 30 % of the global population (Teng et al., 2023). In the new algorithm,

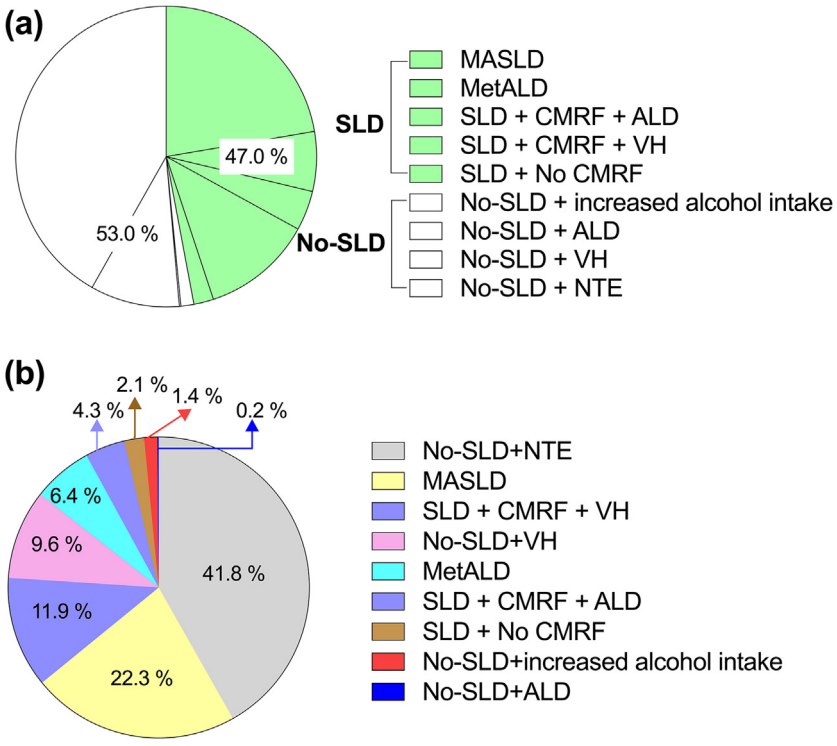

Fig. 6 – The liver disease etiologies underlying significant fibrosis (≥ F2) in NHANES III: 1988–1994. Pie charts show the weighted percentages of the underlying liver disease in people with significant fibrosis (≥ F2, FIB-4 ≥ 2.67). (a) SLD and non-SLD; (b) All major liver diseases sorted by weighted percentages (descending order). VH: viral hepatitis.

the first decision point is steatosis (yes/no). The new algorithm provides a pathway for assigning everyone in a population to a specific liver disease category and clearly defines a category that has received little attention in the past, people without steatosis and without viral hepatitis, alcohol-associated liver disease, or MASLD. We called this category the NTE group. Currently, the criteria for diagnosing steatosis are unspecified (Rinella et al., 2023a). Given this lack of specificity, in this study we used a range of CAP thresholds to define steatosis. As expected, as the CAP threshold increased the number of people in the MASLD category decreased and the number in the NTE category increased. Clinicians, population health, and public health experts need to be aware of this relationship and its implications. When a higher threshold is used, more people are assigned to the NTE category rather than the MASLD category; it is likely that these people will receive less follow-up care and monitoring. Research is needed to evaluate the risks and benefits of using stricter vs. more lenient diagnostic criteria for diagnosing liver steatosis. Additionally, it will be important to design liver disease screening programs that include people in the easily-overlooked NTE category and to fully characterize their liver-related risk factors. Our findings show that these risk factors include heavy metals in NHANES (2017–2020).

The equipment and methods used to diagnose and grade steatosis have changed over time and have increased in sensitivity. VCTE received FDA approval in 2013. Prior to that time, ultrasound, a less sensitive method, was commonly used. To assemble as large a dataset as possible and to look for changes over time, we included both ultrasound-defined and VCTE-CAP-defined steatosis. By using this approach, we were able to include data from NHANES III (1988–1994) and NHANES (2017–2020).

3.2. *Implications of risk factor assessments for liver fibrosis*

Both cardiometabolic and environmental risk factors for liver fibrosis were assessed in the MASLD and NTE groups. Blood Pb in the 4th quartile was a MASLD-fibrosis risk factors in NHB in the earlier NHANES III dataset and was an NTE-fibrosis risk factor in the total population in the more recent NHANES 2017–2020 dataset. In the more recent dataset, participants in the NTE category with 4th quartile blood Pb had a four-fold higher risk of significant fibrosis (≥ F2, liver stiffness ≥ 8.6 kPa) than those with lower Pb levels. Fortunately blood levels of Pb and Cd have declined over time in the United States (Ma et al., 2023a). However, both Cd and Pb have long biological half-lives. Cd can persist for 17 to 30 years, primarily in the kidney and liver (Fatima et al., 2019). Pb is stored in bone and leaches out over time, impacting the liver and other organs and disrupting metabolic functions (Collin et al., 2022). The strong association between Pb and liver fibrosis underscores the vital importance of recent mandates to reduce Pb exposures (US EPA, 2023). Levels remain high in many communities, as illustrated by the health crisis in Flint, Michigan (Hanna-Attisha et al., 2016). Chelation therapy (Teerasarntipan et al., 2020) might be considered as a treatment for people with heavy metal toxicity. Additional treatment options could include compounds reported

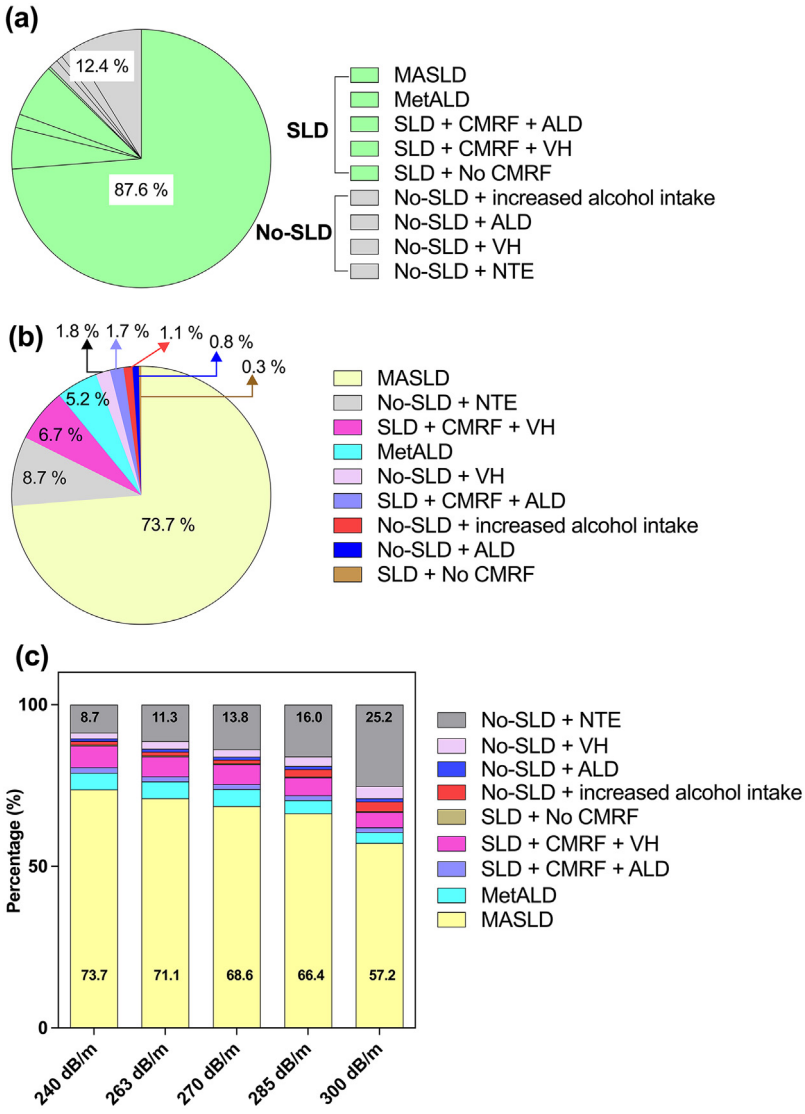

Fig. 7 – The liver disease etiologies underlying significant fibrosis (≥ F2, liver stiffness ≥ 8.6 kPa) in NHANES: 2017-March 2020. Pie charts show the weighted percentages of the underlying liver disease in people with significant fibrosis defined by VCTE liver stiffness ≥ 8.6 kPa, (a) SLD and non-SLD; (b) All liver diseases sorted by weighted percentages (descending order). The stacked bagplot (c) shows the weighted prevalence of the underlying liver diseases in people with significant fibrosis across a range of VCTE-CAP thresholds for the diagnosis of steatosis.

to reduce Cd-related and Pb-related liver damage (Mitra et al., 2022).

3.3. *Racial disparities in liver fibrosis: potential links to genomic differences*

The strong association between Pb exposure and liver fibrosis merits further investigation, especially in NHB. NHB had distinctive risk factors of liver fibrosis. MASLD-fibrosis was associated with 4th quartile blood Pb but was not associated with diabetes or hypertension (two of the five MASLD-defining CMFRs), consistent with earlier findings (Browning et al., 2018; Ma et al., 2023a, 2023b). These results highlight the need to better understand the heterogeneity in the relationship between metabolic parameters and liver disease progression. Genetic factors influence the risk and severity of liver disease across various racial/ethnic groups (Kubiliun et al., 2022; Romeo et al., 2008; Rutledge et al., 2023) and could contribute to differential susceptibility to environmental exposures (Au, 2001; Kosnik et al., 2021).

3.4. *Changes in the liver disease etiologies underlying fibrosis*

A notable finding of our study was that in both datasets, the NTE category had a large percentage of the fibrosis cases: 42 % in the earlier NHANES III dataset (1988–1994) and about 9 %–16 % in the later dataset (NHANES 2017–2020), which was the second largest category, surpassed only by MASLD, which accounted for about 70 % in the later dataset and about 22 %

in the earlier dataset. Many factors likely contribute to the changing proportions of fibrosis cases in the MASLD and NTE categories, including increases in metabolic dysfunction, and increases in ALD (Rinella et al., 2023a). Additionally, some of the differences may be due to differences in methodology.

We found no significant difference in mortality between the MASLD and NTE groups after adjusting for various confounders. This is consistent with a previous report that found no significant difference in all-cause mortality between people with and without NAFLD (Huang et al., 2021). The indistinguishable mortality between the MASLD and NTE categories and the large number of fibrosis cases in the NTE category highlight the importance of screening for liver disease across the entire population. This could be done using an economical and non-invasive approach, as we discussed before (Ma et al., 2023a).

3.5. *Strengths and weaknesses of this study*

The strengths include the use NHANES data, which provide nationally representative information, the use of VCTE, a widely-used methodology to estimate steatosis and fibrosis in the later dataset, and the analysis of mortality with about 30 years of follow. Multiple CAP cut points were used to investigate most end points. The limitations include NHANES' cross-sectional design, the lack of biopsy, MRI or MR elastography data to confirm liver steatosis and liver fibrosis, and the resulting inability to evaluate metabolic dysfunction-associated steatohepatitis (MASH), and the lack of liver stiffness and CAP measurements in the earlier dataset. Additionally, NHANES has limited data about the less common liver diseases, such as autoimmune hepatitis; individuals with these diseases may be misclassified.

4. Conclusions

This study projected the diagnostic algorithm that underlies the new liver disease nomenclature onto the population of the US. The data show that as the stringency of the criterion used to define steatosis increases, more people are moved into a diagnostic category that is rarely considered, herein termed NTE. This group can account for 15 % or more of all cases of fibrosis. New diagnostic tools may be needed to identify these patients. Heavy metals were fibrosis risk factors in the NTE group and should be investigated as causal factors. Additionally, because fibrosis risk factors in patients with MASLD vary by race and do not include diabetes or hypertension in NHB, additional research is needed to clarify the pathways connecting liver fibrosis to metabolic dysfunction in all parts of the multi-racial US population.

Data availability

The data that support the findings of this study are openly available in the National Health and Nutrition Examination Survey website: (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ning Ma: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Meena B. Bansal:** Methodology, Writing – review & editing. **Jaime Chu:** Writing – review & editing, Methodology. **Mark Woodward:** Writing – review & editing, Methodology. **Andrea D. Branch:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition.

Acknowledgments

This work was supported by the Prevent Cancer Foundation (No. PCF 604934), the National Institute for Occupational Safety and Health (Nos. U01OH011489, U01OH012263, and U01 OH012622), and Pfizer 70472597.

Appendix A Supplementary data

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jes.2024.08.027.

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